FORM PTO-1390 (REV. 11-94)

U.S DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

ATTORNEY'S DOCKET NUMBER: 8484-081-999

09/486247

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP98/05110	August 12, 1998	August 20, 1997

TITLE OF INVENTION
PROTEASE-RELATED PROTEIN

APPLICANT(S) FOR DO/EO/US
DEAR *et al.*

Applicant herewith submits to the United States Designated/ Elected Office (DO/EO/US) the following items under 35 U.S.C. 371:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the international Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureaus.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 37(c)(3)).

9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) (unexecuted).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☒ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information:

Return Post Card;
Request for International Application;
Copy of front page of PCT as published;
International Search Report;
Request for Preliminary Examination; and
International Preliminary Examination Report.

INTERNATIONAL APPLICATION NO.
PCT/EP98/05110

09/486247

INTERNATIONAL FILING DATE
August 12, 1998

430 Rec'd PCT/PTO 18 FEB 2000

17. ☒ The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees as follows:

	(1)FOR	(2)NUMBER FILED	(3)NUMBER EXTRA	(4)RATE	(5)CALCULATIONS
CLAIMS	TOTAL CLAIMS	21 - 20	1	X $ 18.00	$ 18.00
	INDEPENDENT CLAIMS	2 - 3	0	X $ 78.00	0.00
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $ 260.00	$ 260.00

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

CHECK ONE BOX ONLY

☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... $ 670

☐ No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... $ 760

☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $970

☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2) to (4) ..... $ 96

☒ Filing with EPO or JPO search report ..... $ 840

Surcharge of $130.00 for furnishing the National fee or oath or declaration later than 20 30 mos. from the earliest claimed priority date (37 CFR 1.492(e)).	$ 840.00
TOTAL OF ABOVE CALCULATIONS	= 1,118.00
Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (Note 37 CFR 1.9, 1.27, 1.28).	- $ 0.00
SUBTOTAL	= 1,118.00
Processing fee of $130.00 for furnishing the English Translation later than 20 30 mos. from the earliest claimed priority date (37 CFR 1.492(f)). 0	+
TOTAL FEES ENCLOSED	$ 1,118.00

a. ☐ A check in the amount of $__ to cover the above fees is enclosed.

b. ☒ Please charge Deposit Account No. 16-1150 in the amount of $ 1,118.00 to cover the above fees. A copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 16-1150. A copy of this sheet is enclosed.

18. ☒ Other instructions

Please calculate the claims after entry of the enclosed Preliminary Amendment.

19. ☒ All correspondence for this application should be mailed to

PENNIE & EDMONDS LLP
1155 AVENUE OF THE AMERICAS
NEW YORK, NEW YORK 10036-2711

20. ☒ All telephone inquiries should be made to (212) 790-2803

Birgit Millauer
NAME

SIGNATURE

For: Laura A. Coruzzi
Laura A. Coruzzi
(Reg. No. 30,742)

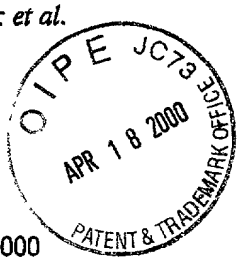
43,341
REGISTRATION NUMBER

February 18, 2000
DATE

P038/K 2783

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: ☒ Application Dear *et al.*
☐ Patent of:



☒ Application No.:
☐ Patent No.:

Group Art Unit: To be assigned

☒ Filed: 18 February 2000
☐ Issued:

Examiner: Herewith

For: PROTEASE-RELATED PROTEIN

Attorney Docket No.:
8484-081-999

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
[37 CFR 1.9(f) and 1.27(d)] - Nonprofit Organization

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of organization Deutsches Krebsforschungszentrum Stiftung des öffentlichen Rechts
Address of organization Im Neuenheimer Feld 280, D-69120 Heidelberg Germany

Type of organization

☐ University or other institution of higher education
☐ Tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3))
☐ Nonprofit scientific or educational under statute of state of the United States of America
(Name of state _____)
(Citation of statute _____)
☒ Would qualify as tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)) if located in the United States of America.
☐ Would qualify as nonprofit scientific or educational under statute of state of the United States of America if located in the United States of America
(Name of state _____)
(Citation of statute _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled PROTEASE-RELATED PROTEIN by inventor(s) Terence Dear and Thomas Boehm described in

☐ the specification filed herewith
☒ application no. filed 18 February 2000
☐ patent no. issued

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization identified above and/or there is an obligation under contract or law by the inventor(s) to convey rights to the nonprofit organization identified above with regard to the invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

FULL NAME_____
ADDRESS_____

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME_____
ADDRESS_____

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28 (b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and patent issuing thereon, or any patent to which this verified statement is directed.

Send correspondence to: PENNIE & EDMONDS LLP
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New York, N.Y. 10036-2711

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Name of person signing	Prof. Dr. med. H. zur Hausen	Dr. rer. pol. J. Puchta
Title of person other than owner	Sc. member of the board	Adm. member of the board
Address of person signing	Eichenstr. 1 69483 Waldmichelbach	Eichenweg 12a 69198 Schriesheim

Signature_____ Date March 30, 2000

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

CA1 - 239415.1

Express Mail No.: EL 451 593 520 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: DEAR *et al.*

Serial No.: To be assigned	Group Art Unit: To be assigned
Filed: HEREWITH	Examiner: To be assigned
For: PROTEASE-RELATED PROTEIN	Attorney Docket No.: 8484-081-999

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In accordance with Rule 111 of the Rules of Practice, 37 C.F.R. § 1.111, please consider and enter the following amendments and remarks.

IN THE CLAIMS:

Please amend the claims as follows:

1. (Amended) A protease-related protein, [the] said protein comprising the amino acid sequence of FIGURE 1 or an amino acid sequence differing therefrom by one or more amino acids.

2. (Amended) A DNA [coding for a] encoding the protein [according to claim] of Claim 1, wherein the DNA comprises:

(a) the DNA of FIGURE 1 or a DNA differing therefrom by one or more base pairs,

(b) a DNA hybridizing with the DNA of (a), or

(c) a DNA related to the DNA of (a) or (b) via the degenerated genetic code.

3. (Amended) An expression plasmid comprising the DNA [according to claim] of Claim 2.

4. (Amended) A transformant [containing] comprising the expression plasmid [according to claim] of Claim 3.

5. (Amended) A process for the preparation of the protein [according to claim] of Claim 1, comprising the cultivation of the transformant [according to claim] of Claim 4 under suitable conditions.

6. (Amended) [Antibodies] An antibody directed against the protein [according to claim] of Claim 1.

7. (Amended) [Use of the protein according to claim 1 and the DNA according to claim 2 as well as the antibody according to claim 6] A method for detecting the keratinization of hair, comprising application the protein of Claim 1, the DNA of Claim 2, or the antibody of Claim 6.

8. (Amended) [Use of the protein according to claim 1] A method for the negative regulation of the keratinization of hair, comprising administering the protein of Claim 1 in a therapeutically effective amount.

9. (Amended) [Use according to claim] The method of Claim 8, wherein the protein is present as such or in the form of a nucleic acid expressing it.

10. (Amended) [Use according to claim] The method of Claim 8 or 9, wherein additionally substances are [also used] administered which inhibit the proteins Ha3 and/or CK15.

11. (Amended) [Use according to claim] The method of Claim 10, wherein the substances are antibodies directed against Ha3 and CK15, respectively, and/or anti-sense

oligonucleotides, all of which inhibit the expression of the nucleic acids encoding these proteins.

12. (Amended) [Use of the protein according to claim 1] A method for the positive regulation of the certification of hair, comprising administering the protein of Claim 1.

13. (Amended) [Use according to claim] The method of Claim 12, wherein the protein is present in the form of a substance inhibiting it.

14. (Amended) [Use according to claim] The method of Claim 13, wherein the substance is an antibody [according to claim] of Claim 6 and/or an anti-sense oligonucleotide which inhibits the expression of the nucleic acid encoding the protein.

15. (Amended) [Use according to any one of claims] The method of Claim 12, 13 [to], or 14, wherein the proteins Ha3 and/or CK15 are also present as such or in the form of nucleic acids expressing them.

REMARKS

The above amendments are made to comply with the formal requirements set forth in 37 C.F.R. §1.75. They do not introduce new matter, and they are fully supported by the specification of the subject Application and the claims as originally filed.

Applicants respectfully request that the above-made amendments be made of record in the file history of the instant application.

Respectfully submitted,

Date February 18, 2000

Birgit Millauer 43,341 (Reg. No.)

For: Laura A. Coruzzi (Reg. No. 30,742)

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Enclosure

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PROTEASE-RELATED PROTEIN

This is a national phase filing of the Application No. PCT/EP98/05110, which was filed with the Patent Corporation Treaty on August 12, 1998, and is entitled to priority of the German Patent Application 197 36 198.6, filed August 20, 1997.

I. FIELD OF THE INVENTION

The present invention relates to a protease-related protein, a DNA encoding the same and a process for the preparation thereof. In addition, this invention concerns the use of the DNA and the protein as well as antibodies directed against the protein.

II. BACKGROUND OF THE INVENTION

A hair anomaly is frequently due to an impaired keratinization of hair. It is known from investigations made with naked mice that the gene product of a gene referred to as whn is important for the keratinization of hair. This gene product is a transcription factor. However, its target genes are not known. In so far, it is not possible to interfere with the keratinization of hair. However, this would be desirable, particularly if the keratinization of the hair is impaired.

Therefore, it is the object of the present invention to provide a product by which the keratinization of hair can be investigated and optionally be regulated.

III. SUMMARY OF THE INVENTION

The present invention relates to a protease-related protein, a DNA encoding the same and a process for the preparation thereof. Furthermore, this invention concerns the use of the DNA and the protein as well as antibodies directed against the protein and antagonistic substances.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the base sequence of a cDNA according to the invention as well as the amino acid sequence, derived therefrom, of a (PVP) according to the invention.

V. DETAILED DESCRIPTION OF THE INVENTION

It is the object of the present invention to provide a product by which the keratinization of hair can be investigated and optionally be regulated. According to the invention this is achieved by the subject matters defined in the claims.

Thus, the subject matter of the present invention is represented by a protease-related protein, the protein comprising the amino acid sequence of FIGURE 1 or an amino acid sequence differing therefrom by one or more amino acids.

The present invention is based on the applicant's finding that the gene product of the whn gene is responsible for regulating the expression of at least three genes. Two of these genes code for the known keratins Ha3 (Winter *et al.*, 1994, *Exp. Cell Res.* 212:190-200) and CK15 (Nozaki *et al.*, 1994, *Gene* 138:197-200), respectively.

The third gene codes for a protein which has homologies with respect to a protease of the kallikrein family, optionally a protease activity, but differs from a known protease of the kallikrein family on the DNA level by hybridization under normal conditions. Such a protein has the amino acid sequence of FIGURE 1 or an amino acid sequence differing therefrom by one or more amino acids. Furthermore, the applicant has found that when the gene product of the whn gene is lacking the genes of Ha3 and CK15 are underexpressed whereas the gene of the above protein is overexpressed.

The above protein is referred to as "protease-related protein" (PVP) in the present invention.

Another subject matter of the present invention relates to a nucleic acid coding for a (PVP). This may be an RNA or a DNA. The latter may be a genomic DNA or a cDNA, for example. Preferred is a DNA comprising the following:

(a) the DNA of FIGURE 1 or a DNA differing therefrom by one or more base pairs,

(b) a DNA hybridizing with the DNA of (a), or

(c) a DNA related to the DNA of (a) or (b) via the degenerated genetic code.

The expression "hybridizing DNA" refers to a DNA which hybridizes with a DNA of (a) under normal conditions, particularly at 20°C below the melting point of the DNA.

A section of the DNA of FIGURE 1 was deposited with the DSMZ *(Deutsche Sammiung von Mikroorganismen und Zelikulturen* [German-type collection of microorganisms and cell cultures]) as pRDA2-1a under DSM 11522 on April 23, 1997.

A DNA according to the invention is described below in the form of a cDNA. It stands as an example for every DNA falling under the present invention.

For the production of a cDNA according to the invention it is favorable to isolate mRNA from skin cells of whn (+/+) mice and nu/nu (whn(-/-)) mice, respectively, to transcribe the mRNA into cDNA and subject the cDNA to a "representational difference analysis" (RDA) method (Hubank and Schatz, 1994, *Nucleic Acids Research* 22:5640-5648) so as to identify that cDNA which is underexpressed and overexpressed, respectively, in nu/nu mice as compared to whn (+/+) mice. In particular the latter cDNA represents a cDNA according to the invention.

A cDNA according to the invention may be present in a-vector and expression vector, respectively. A person skilled in the art is familiar with examples thereof. In the case of an expression vector for *E. coli*, these are, *e.g.,* pGEMEX, pUC derivatives, pGEX-2T, pET3b and pQE-8. For the expression in yeast, pY100 and Ycpad1 have to be mentioned as examples, while for the expression in animal cells, *e.g.,* pKCR, pEFBOS, cDM8 and pCEV4 have to be indicated. For the expression in insect cells, the baculovirus expression vector pAcSGHisNT-A is especially suitable.

The person skilled in the art is familiar with suitable cells to express a cDNA according to the invention, which is present in an expression vector. Examples of such cells comprise the *E. coli* strains HE101, DH1, x1776, JM101, JM 109, BL21 and SG 13009, the yeast strain *saccharomyces cerevisiae* and the animal cells Ltk, 3T3, FM3A, CHO, COS, Vero and HeLa as well as the insect cells sf9.

The person skilled in the art knows in which way a cDNA according to the invention has to be inserted in an expression vector. He is also familiar with the fact that this DNA can be inserted in connection with a DNA coding for another protein and peptide,

respectively, so that the cDNA according to the invention can be expressed in the form of a fusion protein.

In addition, the person skilled in the art is familiar with conditions of cultivating transformed cells and transfected cells, respectively. He also knows processes serving for isolating and purifying the protein expressed by the cDNA according to the invention. Thus, such a protein which may also be a fusion protein represents a subject matter of the present invention as well.

Another subject matter of the present invention relates to an antibody directed against an above protein and fusion-protein, respectively. Such an antibody may be prepared by conventional processes. It may be polyclonal and monoclonal, respectively. For its preparation it is favorable to immunize animals, particularly rabbits or chickens for a polyclonal antibody and mice for a monoclonal antibody, with an above (fusion) protein or fragments thereof. Further boosters of the animals can be effected with the same (fusion) protein or fragments thereof. The polyclonal antibody may then be obtained from the animals' serum and yolk, respectively. For the monoclonal antibody, spleen cells from the animals are fused with myeloma cells.

The present invention enables to investigate the keratinization of hair. (PVP) can be detected in cells, particularly skin cells, by means of an antibody according to the invention. A relation between (PVP) and the keratinization of hair can be established. Moreover, it is possible to detect by means of a (PVP) according to the invention an autoantibody directed against this protein. Both detections may be made by conventional processes, particularly a Western blot, an ELISA, an immunoprecipitation or by immunofluorescence. Furthermore, the expression of the gene coding for (PVP) can be detected by a nucleic acid according to the invention, particularly a DNA and primers derived therefrom. This detection can be carried out as usual, particularly in a Southern blot.

In addition, the present invention is suited to interfere in regulating fashion with the keratinization of hair. This regulation may be positive or negative. A positive regulation is understood to mean one by which an impaired keratinization of hair can be encountered. A negative regulation would exist if a normal or strong keratinization of hair was reduced.

For a positive regulation of the keratinization of hair it is an obvious thing to use (PVP) in the form of a substance inhibiting it. This substance may be an antibody according

to the invention. Furthermore, it may be an anti-sense oligonucleotide which is suited for the expression inhibition of the gene coding for (PVP). Moreover, the substance may be a substance which has an antagonistic effect with respect to (PVP). It may be advantageous to use several substances. It may be especially favorable to use additionally one or more of the proteins Ha3 and CK15 as such or in the form of nucleic acids expressing them.

For a negative regulation of the keratinization of hair, it is an obvious thing to use (PVP) as such or in the form of a nucleic acid expressing it. It may be advantageous to use additionally one or more of the proteins Ha3 and CK15 in the form of substances inhibiting them. Such substances may be antibodies directed against Ha3 and CK15, respectively, or anti-sense oligonucleotides, which are suited for the expression inhibition of the genes coding for Ha3 and CK15, respectively. The substances may also be those which have an antagonistic effect with respect to Ha3 and CK15, respectively.

Another subject matter of the invention relates to a product which is suited for regulating the keratinization of hair. For the composition of such a product the above statements made on the positive and negative regulations of the keratinization of hair apply correspondingly.

Thus, the present invention represents a major contribution to the understanding of the keratinization of hair and a possible regulating interference.

The below examples explain the invention in more detail. The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. The present invention, however, is not limited in scope by the exemplified embodiments, which are intended as illustrations of single aspects of the invention only, and methods which are functionally equivalent are within the scope of the invention. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

VI. EXAMPLES

A. Example 1: Preparation of a cDna According to the Invention

A cDNA according to the invention was prepared according to the "representational difference analysis" (RDA) method. This method comprises the isolation of mRNA from skin cells of whn (+/+) mice and nu/nu mice, respectively, the transcription of the mRNA into cDNA, and the differentiation of the cDNA so as to identify that which is underexpressed and overexpressed, respectively, in nu/nu mice. In particular, the latter represents a cDNA according to the invention.

A) Sequence of the oligonucleotide adaptors

The following oligonucleotide adaptor pairs were required for the RDA:

R-Bgl -12: 5' -GATCTGCGGTGA- 3'
R-Bgl -24: 5' -AGCACTCTCCAGCCTCTCACCGCA -3'

R-Bgl -12: 5' -GATCTGTTCATG -3'
R-Bgl -24: 5' -ACCGACGTCGACTATCCATGAACA -3'

N-Bgl -12: 5' -GATCTTCCCTCG -3'
N-Bgl -24: 5' -AGGCAACTGTGCTATCCGAGGGAA -3'

B) Preparation of poly A-mRNA from the tissues to be compared with one another

RNA was initially obtained from the skin of whn (+/+) mice and nu/nu mice, respectively, according to the "single step RNA extraction" method (Chomczynski and Sacchi, 1987) Thereafter, the poly A-mRNA fractions from the two RNA populations were isolated by means of dynabeads oligo(dT) according to the corresponding protocol from the Dynal company.

C) Synthesis of double-stranded cDNA

The "ribo clone cDNA synthesis kit" from the company of Promega was used for the synthesis of double-stranded whn(+/+) cDNA and nu/nu cDNA, respectively. 4 μg of poly A-mRNA were used each to obtain about 2 μg cDNA.

D) Difference analysis

1. Restriction digest of the double-stranded cDNAs

a) About 2 μg of each cDNA were digested in a 100 μl reaction batch by the restriction endonuclease DpnII at 37°C for 2 h.

b) The reaction solutions were then extracted twice with a phenol/chloroform mixture (1:1) and once with 100 % chloroform.

c) The DNA included in the aqueous phases of the two reaction batches was admixed with 2 μg glycogen, 50 μl 10 M ammonium acetate and 650 μl 100 % ethanol each and precipitated on ice for 20 min.

After 14 minutes of centrifugation at 4°C and with 14000 rpm, the supernatant was discarded and the DNA pellet was washed with 70 % ethanol. After another centrifugation and removal of the alcoholic phase, the dried DNA was resuspended in 20 μl TE buffer.

2. Ligation of the cDNAs to the R-Bgl oligonucleotide adaptor pair

a) A reaction vessel was used to combine the following: 20 μl cut cDNA (total reaction batch from item D) 1c)

8 μg R-Bgl-24

4 μg R-Bgl-12

6 μl 10 x ligase buffer

x μl water

57 μl final volume

b) The reaction batch was heated in a thermocycler (Peltier Thermocycler PTC-200, MJ Research) to 50°C, kept at this temperature for 1 min and then cooled again to 10°C in the course of one hour (ramp rate: 0.1°C/9 sec)

c) After adding 3 μl T4 DNA ligase (1 U/μ1), the mixture was incubated at 16°C overnight.

3. Synthesis of "representations" of the cDNA populations to be compared with one another

a) For generating what is called "representations" of the ligated cDNAs, the volume of the ligation batches from item 2c) was initially supplemented by the addition of 140 μl water each to give 200 μl.

Then, 30 reactions of 200 μl each were prepared from this dilute solution per cDNA population (whn (+/+) skin and nu/nu skin, respectively).

The following reactants were added successively to such a batch:

143 μl water
20 μl 10 x PCR buffer
20 μ 2 mM dNTPs
10 μl 25 mM Mg chloride
2 μl R-Bgl-24 (1 μg/μl)
4 μl dilute ligation batch

b) PCR:
3 min: 72°C
addition of 1 μl Taq-DNA polymerase (5 U/μl)
20 X: 5 min: 95°C
3 min: 72°C
finally: cooling to 4°C.

c) For preparing the reaction solutions, 4 reaction batches each were combined in a vessel.
extraction: 2 x with 700 μl phenol/chloroform (1:1) each, 1 x with chloroform 100 %

precipitation: addition of 75 µl 3 M Na-acetate solution (pH 5.3) and 800 µl 2-propanol to each reaction vessel, 20 min on ice.

centrifugation: 14 min, 14000 rpm, 4°C.

Washing of the DNA pellet with ethanol 70 % and resuspension in such an amount of water that a concentration of 0.5 µg/µl resulted.

4. Restriction digest of the representations"

a) For removing the R-Bgl oligonucleotide adaptors, 300 µg of each representation (whn (+/+) skin and nu/nu skin, respectively) were subjected to a restriction digest. Following the addition of the below reactants, incubation was carried out at 37°C for 4 h:

600 µl cDNA representation (0.5 µg/µl)

140 µl 10 x Dpnll buffer

100 µl Dpnll (10 U/µl)

560 µl water.

b) The restriction digest batch was distributed to 2 vessels prior to its preparation.

Extraction: 2 x phenol/chloroform (1:1), 1 x chloroform 100 %;

precipitation: addition of 70 µl 3 M Na-acetate (pH 5.3), 700 µl 2-propanol to each vessel, 20 min on ice;

centrifugation: 14 min, 14000 rpm, 4°C.

Washing of the DNA pellet with ethanol 70 % and resuspension in such an amount of water that a concentration of 0.5 µg/µl resulted.

The resulting Dpnll-digested whn(+/+) skin-cDNA representation represented the driver-DNA population to be used in the subtractive hybridization.

5. Synthesis of the tester DNA population

a) 20 µg of the nu/nu skin-cDNA representation digested with Dpnll (= tester DNA) were separated electrophoretically in a TAE gel:

40 µl tester DNA (0.5 µg/µl)

50 μl TE buffer
10 μl 10 x loading buffer

were applied to a 1.2 % agarose TAE gel. A voltage was applied to the gel until the bromophenol blue component of the loading buffer had migrated about 2 cm.

b) Thereafter, the bands containing the representation DNA were cut out of the gel and eluted by means of the "agarose gel DNA extraction kit" from the company of Boehringer Mannheim.
The DNA extracts were purified, so that a total of 60 μl of solution was obtained. The concentration of this solution was evaluated by electrophoresis of 5 μl in a 1 % agarose gel.

c) Finally, the tester DNA was ligated with the J oligonucleotide pair:
2 μg tester DNA eluate
6 μl 10 x ligase buffer
4 μl J-Bgl-24 (2 μ/μ1)
4 μl J-Bgl-12 (1 μ/μl)
x μl water
57 μl final volume

d) Transfer of the reaction batch into thermocycler:
1 min: 50°C
cooling to 10°C within 1 h (ramp rate: 0.1°C/9 sec)

e) After adding 3 μl T4 DNA ligase (1 U/μl), incubation at 16°C overnight.

f) Adjusting the concentration of the tester DNA to approximately 10 ng/μl by the addition of 120 μl water.

6. Subtractive hybridization

a) 80 µl of driver DNA (40 µg) from step 4. and 40 µl (0.4 µg) of dilute tester DNA from step 5., ligated with J oligonucleotides, were combined in a reaction vessel and extracted 2 x with phenol/chloroform (1:1) and once with chloroform 100 %.

b) Precipitation by addition of 30 µl of 10 M ammonium acetate, 380 µl of ethanol 100 %; -70°C for 10 min.

Centrifugation: 14 min, 14000 rpm, 4°C.

Then: 2 x washing of the pellet with ethanol 70 %, short centrifugation after every wash step; drying of the DNA pellet.

c) The DNA was resuspended in 4 µl EE x3 buffer (30 mM EPPS, pH 8.0, at 20°C (Sigma company), 3 mMEDTA) - accompanied by pipetting off and on for about 2 min, then heating to 37°C for 5 min, short "vortexing" and finally combining the solution again on the vessel bottom by centrifugation. The solution was eventually covered with a layer consisting of 35 µl of mineral oil.

d) Transfer of the reaction batch into thermocycler:
5 min: 98°C,
cooling to 67°C and immediate addition of 1 µl 5 M NaCl to the DNA, 20 h of incubation at 67°C.

7. Synthesis of the first difference product

a) After removing the mineral oil as completely as possible, the DNA was diluted step-wise:

1. addition of 8 µl TE (+ 5 µg/µl yeast RNA)

2. addition of 25 μl TE - thereafter thorough mixing

3. addition of 362 μl TE - vortex.

b) 4 PCRs were prepared for every subtractive hybridization. Per reaction:

127 μl water

20 μl 10 x buffer

20 μl 2 mM dNTPs

5 μl 25 mM Mg chloride

20 μl dilute hybridization solution(from step 7a)

c) PCR program:

3 min: 72°C

addition of 1 μl Taq DNA polymerase (5 U/μ1)

5 min: 72°C

addition of 2 μl primer J-Bgl-24 (1 μg/μl)

10 x: 1 min: 95°C

3 min: 70°C

finally: 10 min: 72°C, then cooling to room temperature

d) The 4 reaction batches were combined in a 1.5 ml vessel.

extraction: 2 x phenol/chloroform (1:1), 1 x chloroform 100%.

After the addition of 2 μg glycogen carrier:

Precipitation with 75 μl 3 M Na acetate (pH 5.3),

800 μl 2-propanol, 20 min on ice.

Centrifugation: 14 min, 14000 rpm, 4°C.

Washing of the DNA pellet with ethanol 70 %.

After drying of the DNA, resuspension in 40 μl water.

e) 20 μl of the resuspended DNA from

d) were subjected to a "mung bean nuclease digest" (MBN)

20 μl DNA

4 μl 10 x mung bean nuclease buffer (NEB company)

14 μl water

2 μl mung bean nuclease (10 U/μl; NEB company)

35 min, 30°C.

The reaction was discontinued by the addition of 160 μl 50 mM Tris-HC1 (pH 8.9) and 5 minutes of incubation at 98°C. Thereafter, the vessel was placed on ice up to the next step.

f) During the MEN incubation, 4 further PCRs were prepared (on ice) :

127 μl water

20 μl 2 mM dNTPs

10 μl 25 mM Mg chloride

20 μl J-Bgl-24 (1 μg/μl)

20 μl MBN-digested DNA.

g) PCR program:

1 min: 95°C

allowing to cool to 80°C, addition of 1 μl Taq DNA polymerase (5 U/μl), 18 X:

1 min: 95°C

3 min: 70°C,

finally: 10 min: 72°C; allowing to cool to 4°C.

h) The 4 PCR batches were combined in a vessel.

Extraction: 2 x phenol/chloroform (1:1), 1 x chloroform 100 %.

Precipitation: 75 μl 3 M Na-acetate (pH 5.3), 800 μl 2-propanol, 20 min on ice.

Centrifugation: 14 min, 14000 rpm, 4°C.

Washing of the DNA pellet with ethanol 70 %.

Resuspension of the DNA in 100 μl
(resulting concentration: 0.5 μg/μl); solution obtained in this way represented first difference product.

8. Exchange of the oligonucleotide adaptors of the difference product

a) Removal of the oligonucleotide adaptors by restriction digest with DpnII:
40 μl difference product 1 (0.5 μg/μl)
30 μl 10 x DpnII buffer
15 μl DpnII (10 U/μl)
215 μl water
37°C for 2 h.

b) Preparing the reaction batch:
Extraction: 2 x phenol/chloroform (1:1), 1 x chloroform 100%.
Precipitation: 33 μl 3 M Na-acetate (pH 5.3), 800 μl ethanol 100 %, 20 min, - 20°C.
Centrifugation: 14 min, 14000 rpm, 4°C. Washing of the pellet in ethanol 70 % and resuspension in 40 μl water.

c) Ligation of the difference product to N-Bgl oligonucleotide adaptor pair
1 μl of the prepared DNA solution was diluted with 9 μl water concentration of 50 ng/μl; 4 μl of this solution were used in the following reaction:
4 μl DpnII-digested difference product 1 (200 ng)
6 μl 10 x ligase buffer
2.5 μl N-Bgl-24 (3.5 μg/μl)
2 μl N-Bgl-12 (2 μg/μl)
42.5 μl water.

d) After the transfer of the reaction batch into thermocycler:
1 min: 50°C,
allowing to cool to 10°C within one hour (ramp rate: 0.1°C/9 sec)

e) After adding 3 μl T4 DNA ligase (1 μg/μl), incubation at 16°C overnight.

9. Synthesis of the 2nd difference product

The ligation batch from step 8e) was diluted by adding 100 μl water to a concentration of 1.25 ng/μl. 40 μl of this dilution (50 ng) were mixed with 80 μl driver DNA (see item 4.) and treated again according to steps 6. to 8. When the oligonucleotide adaptors (step 8.) were changed, the J-Bgl oligonucleotides were ligated in this case to the newly formed difference product 2.

10. Synthesis of the 3rd difference product

The concentration of the difference product 2 ligated with the J-Bgl oligos was reduced to a concentration of 1 ng/μl. 10 μl of this solution were diluted again with 990 μl water (+ 30 μg yeast-RNA), so that the concentration was then 10 pg/μl). The subtractive hybridization was carried out with 100 pg (10 μl) J-ligated difference product 2 and 40 μg (80 μl) driver DNA from step 4. As for the rest, the procedure was carried out as in the case of the 1st and 2nd difference products according to steps 6. to 8. The PCR according to the MEN reaction (item 7.g) formed an exception - here only 18 cycles in place of 22 ones were carried out.

11. Cloning of the 3rd difference product

The 3rd difference product was initially subjected to a restriction digest with DpnII so as to remove the oligonucleotide adaptors. The reaction product was then applied to a TAE gel and separated electrophoretically. The separated DNA bands were cut out of the gel, the DNA was eluted and cloned into a vector cut with BamHI (pBS Not).

12. Characterization of the difference products

In order to confirm that the cloned DNA fragments were not method artifacts but sequences which were actually included in the investigated DNA representations, Southern analyses were carried out in which the investigated cDNA representations were hybridized with the radioactively labeled cloning products.

Thereafter, those DNA fragments which proved to be "real" difference products in the Southern analysis, were investigated by means of Northern hybridizations: RNAs from the investigated tissues (whn(+/+) skin-cDNA and nu/nu skin-cDNA) were blotted and hybridized with the radioactively labeled cloning products. By this, the differential expression of these sequences was confirmed in the investigated tissues. An analysis of the sequences yielded the cDNA of FIGURE 1 according to the invention.

B. Example 2: Preparation and Purification of a (PVP) According to the Invention

For the preparation of a (PVP) according to the invention, the vector pBSNot-PVP of Example 1 is cleaved by BamHI, the DNA coding for (PVP) is isolated and inserted in the expression vector pQE-8 (Quiagen company) cleaved by BamHI. The expression plasmid pQ/PVP is obtained. Such a plasmid codes for a fusion protein comprising 6 histidine residues (N terminus partner) and the (PVP) of FIGURE 1 according to the invention (C terminus partner). pQ/PVP is used for transforming *E. coli* SG 13009 (Gottesman *et al.*, 1981, *J. Bacteriol.* 148:265-273) . The bacteria are cultivated in an LB broth with 100 μg/ml ampicillin and 25 μg/ml kanamycin and induced with 60 μM isopropyl-1β-D-thiogalactopyranoside (IPTG) for 4 h. The addition of 6 M guanidine hydrochloride serves for achieving lysis of the bacteria, thereafter a chromatography (Ni-NTA resin) is carried out with the lysate in the presence of 8 M urea corresponding to the instructions of the manufacturer (Quiagen company) of the chromatography material. The bound fusion protein is eluted in a buffer having pH 3.5. After its neutralization, the fusion protein is subjected to an 18 % SDS-polyacrylamide gel electrophoresis and dyed with Coomassie blue (Thomas and Kornberg, 1975, *J. Mol. Biol.* 149:709-733). In this way, a (fusion) protein according to the invention can be prepared in highly pure form.

C. Example 3: Preparation and Detection of an Antibody According to the Invention

A fusion protein of Example 1 according to the invention is subjected to an 18% SDS-polyacrylamide gel electrophoresis. After dyeing the gel using 4 M sodium acetate, an about 25 kD long band is cut out of the gel and incubated in phosphate-buffered common salt solution. Gel pieces are sedimented before the protein concentration of the

supernatant is determined by SDS-polyacrylamide gel electrophoresis, which is followed by Coomassie blue dying. Animals are immunized with the gel-purified fusion protein as follows:

Immunization protocol for polyclonal antibodies in rabbits. 35 μg gel-purified fusion protein are used per immunization in 0.7 ml PBS and 0.7 ml complete Freund's adjuvant and incomplete Freund's adjuvant, respectively.

day 0:	1st immunization (complete Freund's adjuvant)
day14:	2nd immunization (incomplete Freund's adjuvant: icFA)
day 28:	3rd immunization (icFA)
day 56:	4th immunization (icFA)
day 80:	bleeding to death

The serum of the rabbit is tested in an immunoblot. For this purpose, a fusion protein of Example 1 according to the invention is subjected to SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter (Khyse-Andersen, 1984, *J. Biochem. Biophys. Meth.* 10:203-209) . The Western blot analysis is carried out as described in Bock *et al.*, 1994, *Virus Genes* 8:215-229. For this purpose, the nitrocellulose filter is incubated with a first antibody at 37°C for 1 h. This antibody is the serum of the rabbit (1:10000 in PBS). After several wash steps using PBS, the nitrocellulose filter is incubated with a second antibody. This antibody is a monoclonal goat anti-rabbit-IgG antibody coupled with alkaline phosphatase (Dianova company) (1:5000) in PBS. A 30-minute incubation at 37°C is followed by several wash steps using PBS and then by the alkaline phosphatase detection reaction using developer solution (36 μM 5'-bromo-4-chloro-3-indolylphosphate, 400 μM nitro blue tetrazolium, 100 mM Tris-HC1, pH 9.5, 100 mM NaC1, 5 mM $MgC1_2$) at room temperature, until bands are visible. In this way, the polyclonal antibodies according to the invention can be prepared.

Immunization protocol for polyclonal antibodies in chickens. 40 μg gel-purified fusion protein are used per immunization in 0.8 ml PBS and 0.8 ml complete Freund's adjuvant and incomplete Freund' s adjuvant, respectively.

day 0: 1st immunization (complete Freund's adjuvant)
day 28: 2nd immunization (incomplete Freund's adjuvant; iCFA)
day 50: 3rd immunization (iCFA)

Antibodies are extracted from yolk and tested in a Western blot. Polyclonal antibodies according to the invention are detected in this way. Immunization protocol for monoclonal mouse antibodies12 μg gel-purified fusion protein are used per immunization in 0.25 ml PBS and 0.25 ml complete Freund's adjuvant and incomplete Freund's adjuvant, respectively. The fusion protein was dissolved in 0.5 ml (without adjuvant) in the 4th immunization.

day 0: 1st immunization (complete Freund's adjuvant)
day 28: 2nd immunization (incomplete Freund's adjuvant; iCFA)
day 56: 3rd immunization (icFA) day 84:4th immunization (PBS)
day 87: fusion

Supernatants of hybridomas are tested in a Western blot. Monoclonal antibodies according to the invention are detected in this way.

All references cited within the body of the instant specification are hereby incorporated by reference in their entirety.

CLAIMS

WHAT IS CLAIMED:

1. A protease-related protein, the protein comprising the amino acid sequence of FIGURE 1 or an amino acid sequence differing therefrom by one or more amino acids.

2. A DNA coding for a protein according to claim 1, wherein the DNA comprises:

(a) the DNA of FIGURE 1 or a DNA differing therefrom by one or more base pairs,

(b) a DNA hybridizing with the DNA of (a), or

(c) a DNA related to the DNA of (a) or (b) via the degenerated genetic code.

3. An expression plasmid comprising the DNA according to claim 2.

4. A transformant containing the expression plasmid according to claim 3.

5. A process for the preparation of the protein according to claim 1, comprising the cultivation of the transformant according to claim 4 under suitable conditions.

6. Antibodies directed against the protein according to claim 1.

7. Use of the protein according to claim 1 and the DNA according to claim 2 as well as the antibody according to claim 6 for detecting the keratinization of hair.

8. Use of the protein according to claim 1 for the negative regulation of the keratinization of hair.

9. Use according to claim 8, wherein the protein is present as such or in the form of a nucleic acid expressing it.

10. Use according to claim 8 or 9, wherein substances are also used which inhibit the proteins Ha3 and/or CK15.

11. Use according to claim 10, wherein the substances are antibodies directed against Ha3 and CK15, respectively, and/or anti-sense oligonucleotides, all of which inhibit the expression of the nucleic acids encoding these proteins.

12. Use of the protein according to claim 1 for the positive regulation of the certification of hair.

13. Use according to claim 12, wherein the protein is present in the form of a substance inhibiting it.

14. Use according to claim 13, wherein the substance is an antibody according to claim 6 and/or an anti-sense oligonucleotide which inhibits the expression of the nucleic acid encoding the protein.

15. Use according to any one of claims 12 to 14, wherein the proteins Ha3 and/or CK15 are also present as such or in the form of nucleic acids expressing them.

ABSTRACT

The present invention relates to a protease-related protein, a DNA encoding the same and a process for the preparation thereof. Furthermore, this invention concerns the use of the DNA and the protein as well as antibodies directed against the protein and antagonistic substances.

```
5'- TAG GTG GTG TCA TTC CCC TCC AAC CTG AGT GCT GGC AGG TAC  42

                                M   P   M   K   M   L   T   M   8
ACT GCT GGC CAC CAG CAG ATG CCC ATG AAG ATG CTG ACA ATG      84

 K   M   L   A   L   C   L   V   L   A   K   S   A   W   22
AAG ATG CTG GCC CTG TGC TTG GTT CTT GCT AAA TCA GCC TGG     126

 S   E   E   Q   E   K   V   V   H   G   G   P   C   L   36
TCG GAG GAA CAG GAG AAG GTG GTT CAT GGA GGC CCG TGT TTG     168

 K   D   S   H   P   F   Q   A   A   L   Y   T   S   G   50
AAG GAC TCC CAC CCT TTC CAG GCT GCC CTC TAC ACC TCA GGT     210

 H   L   L   C   G   G   V   L   I   D   P   Q   W   V   64
CAC TTG CTG TGT GGT GGG GTC CTC ATT GAC CCA CAG TGG GTG     252

 L   T   A   A   H   C   K   K   P   N   L   Q   V   I   78
CTG ACA GCT GCC CAC TGC AAA AAA CCG AAT CTG CAG GTG ATC     294

 L   G   K   H   N   L   R   Q   T   E   T   F   Q   R   92
TTG GGG AAA CAC AAC CTA CGG CAA ACA GAG ACT TTC CAA AGG     336

 Q   I   S   V   D   R   T   I   V   H   P   R   Y   N  106
CAA ATC TCA GTG GAC AGG ACT ATT GTC CAT CCC CGC TAC AAC     378

 P   E   T   H   D   N   D   I   M   M   V   H   L   K  120
CCT GAA ACC CAC GAC AAT GAC ATC ATG ATG GTG CAT CTG AAA     420

 N   P   V   K   F   S   K   K   I   Q   P   L   P   L  134
AAT CCA GTC AAA TTC TCT AAA AAG ATC CAG CCT CTG CCC TTG     462

 K   N   D   C   S   E   E   N   P   N   C   Q   I   L  148
AAG AAT GAC TGC TCT GAG GAG AAT CCC AAC TGC CAG ATC CTG     504

 G   W   G   K   M   E   N   G   D   F   P   D   T   I  162
GGC TGG GGC AAG ATG GAA AAT GGT GAC TTC CCA< GAT ACC ATT    546

 Q   C   A   D   V   H   L   V   P   R   E   Q   C   E  176
CAG TGT GCT GAT GTC CAT CTG GTG CCC CGG GAG CAG TGT GAG     588

 R   A   Y   P   G   K   I   T   Q   S   M   V   C   A  190
CGT GCC TAC CCT GGC AAG ATC ACC CAG AGC ATG GTG TGC GCA     630

 G   D   M   K   E   G   N   D   S   C   Q   G   D   S  204
GGC GAC ATG AAA GAA GGC AAC GAT TCC TGT CAG GGT GAT TCT     672

 G   G   P   L   V   C   G   G   R   L   R   G   L   V  218
GGA GGT CCC CTA GTA TGT GGG GGT CGC CTC CGA GGG CTC GTG     714
```

Fig. 1

Fig. 1 continued

S	**W**	**G**	**D**	**M**	**P**	**C**	**G**	**S**	**K**	**E**	**K**	**P**	**G**	232
TCA	TGG	GGT	GAC	ATG	CCC	TGT	GGA	TCA	AAG	GAG	AAG	CCA	GGA	756
V	**Y**	**T**	**D**	**V**	**C**	**T**	**H**	**I**	**R**	**W**	**I**	**Q**	**N**	246
GTT	TAC	ACC	GAT	GTC	TGC	ACT	CAT	ATC	AGA	TGG	ATC	CAA	AAC	798
I	**L**	**R**	**N**	**K**	**W**	**L**								253
ATC	CTC	AGA	AAC	AAG	TGG	CTG	TGA	-3′						840

Protease-Related Protein

The present invention relates to a protease-related protein, a DNA encoding the same and a process for the preparation thereof. In addition, this invention concerns the use of the DNA and the protein as well as antibodies directed against the protein.

A hair anomaly is frequently due to an impaired keratinization of hair. It is known from investigations made with naked mice that the gene product of a gene referred to as whn is important for the keratinization of hair. This gene product is a transcription factor. However, its target genes are not known. In so far, it is not possible to interfere with the keratinization of hair. However, this would be desirable, particularly if the keratinization of the hair is impaired.

Therefore, it is the object of the present invention to provide a product by which the keratinization of hair can be investigated and optionally be regulated.

According to the invention this is achieved by the subject matters defined in the claims.

Thus, the subject matter of the present invention is represented by a protease-related protein, the protein comprising the amino acid sequence of fig. 1 or an amino acid sequence differing therefrom by one or more amino acids.

The present invention is based on the applicant's finding that the gene product of the whn gene is responsible for regulating the expression of at least three genes. Two of these genes code for the known keratins Ha3 (cf. Winter, H. et al., Exp. Cell Res. 212 (1994), 190-200) and CK15 (cf. Nozaki, M. et al., Gene 138 (1994), 197-200), respectively.

The third gene codes for a protein which has homologies with respect to a protease of the kallikrein family, optionally a protease activity, but differs from a known protease of the kallikrein family on the DNA level by hybridization under normal conditions. Such a protein has the amino acid sequence of fig. 1 or an amino acid sequence differing therefrom by one or more amino acids. Furthermore, the applicant has found that when the gene product of the whn gene is lacking the genes of Ha3 and CK15 are underexpressed whereas the gene of the above protein is overexpressed.

The above protein is referred to as "protease-related protein" (PVP) in the present invention.

Another subject matter of the present invention relates to a nucleic acid coding for a (PVP). This may be an RNA or a DNA. The latter may be a genomic DNA or a cDNA, for example. Preferred is a DNA comprising the following:

(a) the DNA of fig. 1 or a DNA differing therefrom by one or more base pairs,
(b) a DNA hybridizing with the DNA of (a), or
(c) a DNA related to the DNA of (a) or (b) via the degenerated genetic code.

The expression "hybridizing DNA" refers to a DNA which hybridizes with a DNA of (a) under normal conditions, particularly at 20°C below the melting point of the DNA.

A section of the DNA of fig. 1 was deposited with the DSMZ (*Deutsche Sammlung von Mikroorganismen und Zellkulturen* [German-type collection of microorganisms and cell cultures]) as pRDA2-1a under DSM 11522 on April 23, 1997.

A DNA according to the invention is described below in the form of a cDNA. It stands as an example for every DNA falling under the present invention.

For the production of a cDNA according to the invention it is favorable to isolate mRNA from skin cells of whn(+/+) mice and nu/nu(whn(-/-)) mice, respectively, to transcribe the mRNA into cDNA and subject the cDNA to a "representational difference analysis" (RDA) method (cf. Hubank, M. and Schatz, D., Nucleic Acids Research 22 (1994), 5640-5648) so as to identify that cDNA which is underexpressed and overexpressed, respectively, in nu/nu mice as compared to whn(+/+) mice. In particular the latter cDNA represents a cDNA according to the invention.

A cDNA according to the invention may be present in a vector and expression vector, respectively. A person skilled in the art is familiar with examples thereof. In the case of an expression vector for E. coli, these are e.g. pGEMEX, pUC derivatives, pGEX-2T, pET3b and pQE-8. For the expression in yeast, pY100 and Ycpad1 have to be mentioned as examples, while for the expression in animal cells, e.g. pKCR, pEFBOS, cDM8 and pCEV4 have to be indicated. For the expression in insect cells, the baculo virus expression vector pAcSGHisNT-A is especially suitable.

The person skilled in the art is familiar with suitable cells to express a cDNA according to the invention, which is present in an expression vector. Examples of such cells comprise the E. coli strains HB101, DH1, x1776, JM101, JM 109, BL21 and SG 13009, the yeast strain *saccharomyces cerevisiae* and the animal cells Ltk, 3T3, FM3A, CHO, COS, Vero and HeLa as well as the insect cells sf9.

The person skilled in the art knows in which way a cDNA according to the invention has to be inserted in an expression vector. He is also familiar with the fact that this DNA can be inserted in connection with a DNA coding for another protein and peptide, respectively, so that the

cDNA according to the invention can be expressed in the form of a fusion protein.

In addition, the person skilled in the art is familiar with conditions of cultivating transformed cells and transfected cells, respectively. He also knows processes serving for isolating and purifying the protein expressed by the cDNA according to the invention. Thus, such a protein which may also be a fusion protein represents a subject matter of the present invention as well.

Another subject matter of the present invention relates to an antibody directed against an above protein and fusion protein, respectively. Such an antibody may be prepared by conventional processes. It may be polyclonal and monoclonal, respectively. For its preparation it is favorable to immunize animals, particularly rabbits or chickens for a polyclonal antibody and mice for a monoclonal antibody, with an above (fusion) protein or fragments thereof. Further boosters of the animals can be effected with the same (fusion) protein or fragments thereof. The polyclonal antibody may then be obtained from the animals' serum and yolk, respectively. For the monoclonal antibody, spleen cells from the animals are fused with myeloma cells.

The present invention enables to investigate the keratinization of hair. (PVP) can be detected in cells, particularly skin cells, by means of an antibody according to the invention. A relation between (PVP) and the keratinization of hair can be established. Moreover, it is possible to detect by means of a (PVP) according to the invention an autoantibody directed against this protein. Both detections may be made by conventional processes, particularly a Western blot, an ELISA, an immunoprecipitation or by immunofluorescence. Furthermore, the expression of the gene coding for (PVP) can be detected by a nucleic acid according to the invention, particularly

a DNA and primers derived therefrom. This detection can be carried out as usual, particularly in a Southern blot.

In addition, the present invention is suited to interfere in regulating fashion with the keratinization of hair. This regulation may be positive or negative. A positive regulation is understood to mean one by which an impaired keratinization of hair can be encountered. A negative regulation would exist if a normal or strong keratinization of hair was reduced.

For a positive regulation of the keratinization of hair it is an obvious thing to use (PVP) in the form of a substance inhibiting it. This substance may be an antibody according to the invention. Furthermore, it may be an anti-sense oligonucleotide which is suited for the expression inhibition of the gene coding for (PVP). Moreover, the substance may be a substance which has an antagonistic effect with respect to (PVP). It may be advantageous to use several substances. It may be especially favorable to use additionally one or more of the proteins Ha3 and CK15 as such or in the form of nucleic acids expressing them.

For a negative regulation of the keratinization of hair, it is an obvious thing to use (PVP) as such or in the form of a nucleic acid expressing it. It may be advantageous to use additionally one or more of the proteins Ha3 and CK15 in the form of substances inhibiting them. Such substances may be antibodies directed against Ha3 and CK15, respectively, or anti-sense oligonucleotides, which are suited for the expression inhibition of the genes coding for Ha3 and CK15, respectively. The substances may also be those which have an antagonistic effect with respect to Ha3 and CK15, respectively.

Another subject matter of the invention relates to a product which is suited for regulating the keratinization of hair. For the composition of such a product the above

statements made on the positive and negative regulations of the keratinization of hair apply correspondingly.

Thus, the present invention represents a major contribution to the understanding of the keratinization of hair and a possible regulating interference.

Brief description of the drawing:

Fig. 1 shows the base sequence of a cDNA according to the invention as well as the amino acid sequence, derived therefrom, of a (PVP) according to the invention.

The present invention is explained by the below examples.

Example 1: Preparation of a cDNA according to the invention

A cDNA according to the invention was prepared according to the "representational difference analysis" (RDA) method. This method comprises the isolation of mRNA from skin cells of whn(+/+) mice and nu/nu mice, respectively, the transcription of the mRNA into cDNA, and the differentiation of the cDNA so as to identify that which is underexpressed and overexpressed, respectively, in nu/nu mice. In particular, the latter represents a cDNA according to the invention.

A) Sequence of the oligonucleotide adaptors

The following oligonucleotide adaptor pairs were required for the RDA:

R-Bgl-12: 5'-GATCTGCGGTGA-3'
R-Bgl-24: 5'-AGCACTCTCCAGCCTCTCACCGCA-3'

R-Bgl-12: 5'-GATCTGTTCATG-3'
R-Bgl-24: 5'-ACCGACGTCGACTATCCATGAACA-3'

N-Bgl-12: 5'-GATCTTCCCTCG-3'
N-Bgl-24: 5'-AGGCAACTGTGCTATCCGAGGGAA-3'

B) Preparation of poly A-mRNA from the tissues to be compared with one another

RNA was initially obtained from the skin of whn(+/+) mice and nu/nu mice, respectively, according to the "single step RNA extraction" method (Chomczynski and Sacchi, 1987). Thereafter, the poly A-mRNA fractions from the two RNA populations were isolated by means of dynabeads oligo(dT) according to the corresponding protocol from the Dynal company.

C) Synthesis of double-stranded cDNA

The "ribo clone cDNA synthesis kit" from the company of Promega was used for the synthesis of double-stranded whn(+/+) cDNA and nu/nu cDNA, respectively. 4 µg of poly A-mRNA were used each to obtain about 2 µg cDNA.

D) Difference analysis

1. Restriction digest of the double-stranded cDNAs

   a) About 2 µg of each cDNA were digested in a 100 µl reaction batch by the restriction endonuclease Dpnll at 37°C for 2 h.

   b) The reaction solutions were then extracted twice with a phenol/chloroform mixture (1:1) and once with 100 % chloroform.

   c) The DNA included in the aqueous phases of the two reaction batches was admixed with 2 µg glycogen,

50 µl 10 M ammonium acetate and 650 µl 100 % ethanol each and precipitated on ice for 20 min.

After 14 minutes of centrifugation at 4°C and with 14000 rpm, the supernatant was discarded and the DNA pellet was washed with 70 % ethanol. After another centrifugation and removal of the alcoholic phase, the dried DNA was resuspended in 20 µl TE buffer.

2. Ligation of the cDNAs to the R-Bgl oligonucleotide adaptor pair

a) A reaction vessel was used to combine the following:
20 µl cut cDNA (total reaction batch from item D)1c)
8 µg R-Bgl-24
4 µg R-Bgl-12
6 µl 10 x ligase buffer
x µl water
57 µl final volume

b) The reaction batch was heated in a thermocycler (Peltier Thermocycler PTC-200, MJ Research) to 50°C, kept at this temperature for 1 min and then cooled again to 10°C in the course of one hour (ramp rate: 0.1°C/9 sec).

c) After adding 3 µl T4 DNA ligase (1 U/µl), the mixture was incubated at 16°C overnight.

3. Synthesis of "representations" of the cDNA populations to be compared with one another

a) For generating what is called "representations" of the ligated cDNAs, the volume of the ligation batches from item 2c) was initially supplemented

by the addition of 140 μl water each to give 200 μl.

Then, 30 reactions of 200 μl each were prepared from this dilute solution per cDNA population (whn(+/+) skin and nu/nu skin, respectively).

The following reactants were added successively to such a batch:
143 μl water
20 μl 10x PCR buffer
20 μl 2 mM dNTPs
10 μl 25 mM Mg chloride
2 μl R-Bgl-24 (1 μg/μl)
4 μl dilute ligation batch

b) PCR:
3 min: 72°C
addition of 1 μl Taq-DNA polymerase (5 U/μl)
20 x: 5 min: 95°C
3 min: 72°C
finally: cooling to 4°C.

c) For preparing the reaction solutions, 4 reaction batches each were combined in a vessel.
extraction: 2 x with 700 μl phenol/chloroform (1:1) each, 1 x with chloroform 100 %;
precipitation: addition of 75 μl 3 M Na-acetate solution (pH 5.3) and 800 μl 2-propanol to each reaction vessel, 20 min on ice.
centrifugation: 14 min, 14000 rpm, 4°C.
Washing of the DNA pellet with ethanol 70 % and resuspension in such an amount of water that a concentration of 0.5 μg/μl resulted.

4. Restriction digest of the "representations"

a) For removing the R-Bgl oligonucleotide adaptors, 300 µg of each representation (whn(+/+) skin and nu/nu skin, respectively) were subjected to a restriction digest. Following the addition of the below reactants, incubation was carried out at 37°C for 4 h:
600 µl cDNA representation (0.5 µg/µl)
140 µl 10 x DpnII buffer
100 µl DpnII (10 U/µl)
560 µl water.

b) The restriction digest batch was distributed to 2 vessels prior to its preparation.
Extraction: 2 x phenol/chloroform (1:1), 1 x chloroform 100 %;
precipitation: addition of 70 µl 3 M Na-acetate (pH 5.3), 700 µl 2-propanol to each vessel, 20 min on ice;
centrifugation: 14 min, 14000 rpm, 4°C.
Washing of the DNA pellet with ethanol 70 % and resuspension in such an amount of water that a concentration of 0.5 µg/µl resulted.

The resulting DpnII-digested whn(+/+) skin-cDNA representation represented the driver-DNA population to be used in the subtractive hybridization.

5. Synthesis of the tester DNA population

a) 20 µg of the nu/nu skin-cDNA representation digested with DpnII (= tester DNA) were separated electrophoretically in a TAE gel:
40 µl tester DNA (0.5 µg/µl)
50 µl TE buffer
10 µl 10 x loading buffer

were applied to a 1.2 % agarose TAE gel. A voltage was applied to the gel until the bromophenol blue component of the loading buffer had migrated about 2 cm.

b) Thereafter, the bands containing the representation DNA were cut out of the gel and eluted by means of the "agarose gel DNA extraction kit" from the company of Boehringer Mannheim.

The DNA extracts were purified, so that a total of 60 µl of solution was obtained. The concentration of this solution was evaluated by electrophoresis of 5 µl in a 1 % agarose gel.

c) Finally, the tester DNA was ligated with the J oligonucleotide pair:
2 µg tester DNA eluate
6 µl 10 x ligase buffer
4 µl J-Bgl-24 (2 µg/µl)
4 µl J-Bgl-12 (1 µg/µl)
x µl water
57 µl final volume

d) Transfer of the reaction batch into thermocycler:
1 min: 50°C
cooling to 10°C within 1 h (ramp rate: 0.1°C/9 sec).

e) After adding 3 µl T4 DNA ligase (1 U/µl), incubation at 16°C overnight.

f) Adjusting the concentration of the tester DNA to approximately 10 ng/µl by the addition of 120 µl water.

6. Subtractive hybridization

a) 80 µl of driver DNA (40 µg) from step 4. and 40 µl (0.4 µg) of dilute tester DNA from step 5., ligated with J oligonucleotides, were combined in a reaction vessel and extracted 2 x with phenol/chloroform (1:1) and once with chloroform 100 %.

b) Precipitation by addition of 30 µl of 10 M ammonium acetate, 380 µl of ethanol 100 %; -70°C for 10 min.

Centrifugation:	14 min, 14000 rpm, 4°C.
Then:	2 x washing of the pellet with ethanol 70 %, short centrifugation after every wash step; drying of the DNA pellet.

c) The DNA was resuspended in 4 µl EE x3 buffer (30 mM EPPS, pH 8.0, at 20°C (Sigma company), 3 mM EDTA) - accompanied by pipetting off and on for about 2 min, then heating to 37°C for 5 min, short "vortexing" and finally combining the solution again on the vessel bottom by centrifugation. The solution was eventually covered with a layer consisting of 35 µl of mineral oil.

d) Transfer of the reaction batch into thermocycler:
5 min: 98°C,
cooling to 67°C and immediate addition of 1 µl 5 M NaCl to the DNA,
20 h of incubation at 67°C.

7. Synthesis of the first difference product

a) After removing the mineral oil as completely as possible, the DNA was diluted step-wise:
   1. addition of 8 µl TE (+ 5 µg/µl yeast RNA)
   2. addition of 25 µl TE - thereafter thorough mixing
   3. addition of 362 µl TE - vortex.

b) 4 PCRs were prepared for every subtractive hybridization. Per reaction:
   127 µl water
   20 µl 10 x buffer
   20 µl 2 mM dNTPs
   5 µl 25 mM Mg chloride
   20 µl dilute hybridization solution (from step 7a))

c) PCR program:
   3 min: 72°C
   addition of 1 µl Taq DNA polymerase (5 U/µl)
   5 min: 72°C
   addition of 2 µl primer J-Bgl-24 (1 µg/µl)
   10 x: 1 min: 95°C
   3 min: 70°C
   finally: 10 min: 72°C, then cooling to room temperature

d) The 4 reaction batches were combined in a 1.5 ml vessel.
   extraction: 2 x phenol/chloroform (1:1), 1 x chloroform 100%.
   After the addition of 2 µg glycogen carrier:
   Precipitation with 75 µl 3 M Na acetate (pH 5.3), 800 µl 2-propanol, 20 min on ice.
   Centrifugation: 14 min, 14000 rpm, 4°C.
   Washing of the DNA pellet with ethanol 70 %.

After drying of the DNA, resuspension in 40 µl water.

e) 20 µl of the resuspended DNA from d) were subjected to a "mung bean nuclease digest" (MBN):
20 µl DNA
4 µl 10 x mung bean nuclease buffer (NEB company)
14 µl water
2 µl mung bean nuclease (10 U/µl; NEB company)
35 min, 30°C.

The reaction was discontinued by the addition of 160 µl 50 mM Tris-HCl (pH 8.9) and 5 minutes of incubation at 98°C. Thereafter, the vessel was placed on ice up to the next step.

f) During the MBN incubation, 4 further PCRs were prepared (on ice):
127 µl water
20 µl 2 mM dNTPs
10 µl 25 mM Mg chloride
2 µl J-Bgl-24 (1 µg/µl)
20 µl MBN-digested DNA.

g) PCR program:
1 min: 95°C
allowing to cool to 80°C, addition of 1 µl Taq DNA polymerase (5 U/µl),
18 x: 1 min: 95°C
3 min: 70°C,
finally: 10 min: 72°C; allowing to cool to 4°C.

h) The 4 PCR batches were combined in a vessel.
Extraction: 2 x phenol/chloroform (1:1), 1 x chloroform 100 %.
Precipitation: 75 µl 3 M Na-acetate (pH 5.3), 800 µl 2-propanol, 20 min on ice.
Centrifugation: 14 min, 14000 rpm, 4°C.

Washing of the DNA pellet with ethanol 70 %.
Resuspension of the DNA in 100 µl water (resulting concentration: 0.5 µg/µl); the solution obtained in this way represented the first difference product.

8. Exchange of the oligonucleotide adaptors of the difference product

a) Removal of the oligonucleotide adaptors by restriction digest with DpnII:
40 µl difference product 1 (0.5 µg/µl)
30 µl 10 x DpnII buffer
15 µl DpnII (10 U/µl)
215 µl water

37°C for 2 h.

b) Preparing the reaction batch:
Extraction: 2 x phenol/chloroform (1:1), 1 x chloroform 100 %.
Precipitation: 33 µl 3 M Na-acetate (pH 5.3), 800 µl ethanol 100 %, 20 min, -20°C.
Centrifugation: 14 min, 14000 rpm, 4°C.
Washing of the pellet in ethanol 70 % and resuspension in 40 µl water.

c) Ligation of the difference product to N-Bgl oligonucleotide adaptor pair
1 µl of the prepared DNA solution from step b) was diluted with 9 µl water to give a concentration of 50 ng/µl; 4 µl of this solution were used in the following reaction:
4 µl DpnII-digested difference product 1 (200 ng)
6 µl 10 x ligase buffer
2.5 µl N-Bgl-24 (3.5 µg/µl)
2 µl N-Bgl-12 (2 µg/µl)
42.5 µl water.

d) After the transfer of the reaction batch into thermocycler:
1 min: 50°C,
allowing to cool to 10°C within one hour (ramp rate: 0.1°C/9 sec).

e) After adding 3 µl T4 DNA ligase (1 µg/µl), incubation at 16°C overnight.

9. Synthesis of the 2nd difference product

The ligation batch from step 8e) was diluted by adding 100 µl water to a concentration of 1.25 ng/µl. 40 µl of this dilution (50 ng) were mixed with 80 µl driver DNA (see item 4.) and treated again according to steps 6. to 8. When the oligonucleotide adaptors (step 8.) were changed, the J-Bgl oligonucleotides were ligated in this case to the newly formed difference product 2.

10. Synthesis of the 3rd difference product

The concentration of the difference product 2 ligated with the J-Bgl oligos was reduced to a concentration of 1 ng/µl. 10 µl of this solution were diluted again with 990 µl water (+ 30 µg yeast-RNA), so that the concentration was then 10 pg/µl. The subtractive hybridization was carried out with 100 pg (10 µl) J-ligated difference product 2 and 40 µg (80 µl) driver DNA from step 4. As for the rest, the procedure was carried out as in the case of the 1st and 2nd difference products according to steps 6. to 8. The PCR according to the MBN reaction (item 7.g) formed an exception - here only 18 cycles in place of 22 ones were carried out.

11. Cloning of the 3rd difference product

The 3rd difference product was initially subjected to a restriction digest with DpnII so as to remove the oligonucleotide adaptors. The reaction product was then applied to a TAE gel and separated electrophoretically. The separated DNA bands were cut out of the gel, the DNA was eluted and cloned into a vector cut with BamHI (pBS Not).

12. Characterization of the difference products

In order to confirm that the cloned DNA fragments were not method artifacts but sequences which were actually included in the investigated DNA representations, Southern analyses were carried out in which the investigated cDNA representations were hybridized with the radioactively labeled cloning products.

Thereafter, those DNA fragments which proved to be "real" difference products in the Southern analysis, were investigated by means of Northern hybridizations: RNAs from the investigated tissues (whn(+/+) skin-cDNA and nu/nu skin-cDNA) were blotted and hybridized with the radioactively labeled cloning products. By this, the differential expression of these sequences was confirmed in the investigated tissues. An analysis of the sequences yielded the cDNA of fig. 1 according to the invention.

Example 2: Preparation and purification of a (PVP) according to the invention

For the preparation of a (PVP) according to the invention, the vector pBSNot-PVP of Example 1 is cleaved by BamHI, the DNA coding for (PVP) is isolated and inserted in the expression vector pQE-8 (Quiagen company) cleaved by BamHI. The expression plasmid pQ/PVP is obtained. Such a plasmid

codes for a fusion protein comprising 6 histidine residues (N terminus partner) and the (PVP) of fig. 1 according to the invention (C terminus partner). pQ/PVP is used for transforming E. coli SG 13009 (cf. Gottesman, S. et al., J. Bacteriol. 148, (1981), 265-273). The bacteria are cultivated in an LB broth with 100 µg/ml ampicillin and 25 µg/ml kanamycin and induced with 60 µM isopropyl-ß-D-thiogalactopyranoside (IPTG) for 4 h. The addition of 6 M guanidine hydrochloride serves for achieving lysis of the bacteria, thereafter a chromatography (Ni-NTA resin) is carried out with the lysate in the presence of 8 M urea corresponding to the instructions of the manufacturer (Quiagen company) of the chromatography material. The bound fusion protein is eluted in a buffer having pH 3.5. After its neutralization, the fusion protein is subjected to an 18 % SDS-polyacrylamide gel electrophoresis and dyed with Coomassie blue (cf. Thomas, J.O. and Kornberg, R.D., J. Mol. Biol. 149 (1975), 709-733).

In this way, a (fusion) protein according to the invention can be prepared in highly pure form.

Example 3: Preparation and detection of an antibody according to the invention

A fusion protein of Example 1 according to the invention is subjected to an 18 % SDS-polyacrylamide gel electrophoresis. After dyeing the gel using 4 M sodium acetate, an about 25 kD long band is cut out of the gel and incubated in phosphate-buffered common salt solution. Gel pieces are sedimented before the protein concentration of the supernatant is determined by SDS-polyacrylamide gel electrophoresis, which is followed by Coomassie blue dyeing. Animals are immunized with the gel-purified fusion protein as follows:

Immunization protocol for polyclonal antibodies in rabbits

35 µg gel-purified fusion protein are used per immunization in 0.7 ml PBS and 0.7 ml complete Freund's adjuvant and incomplete Freund's adjuvant, respectively.

day 0: 1st immunization (complete Freund's adjuvant)
day 14: 2nd immunization (incomplete Freund's adjuvant: icFA)
day 28: 3rd immunization (icFA)
day 56: 4th immunization (icFA)
day 80: bleeding to death

The serum of the rabbit is tested in an immunoblot. For this purpose, a fusion protein of Example 1 according to the invention is subjected to SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter (cf. Khyse-Andersen, J., J. Biochem. Biophys. Meth. 10 (1984), 203-209). The Western blot analysis is carried out as described in Bock, C.-T. et al., Virus Genes 8 (1994), 215-229). For this purpose, the nitrocellulose filter is incubated with a first antibody at 37°C for 1 h. This antibody is the serum of the rabbit (1:10000 in PBS). After several wash steps using PBS, the nitrocellulose filter is incubated with a second antibody. This antibody is a monoclonal goat anti-rabbit-IgG antibody coupled with alkaline phosphatase (Dianova company) (1:5000) in PBS. A 30-minute incubation at 37°C is followed by several wash steps using PBS and then by the alkaline phosphatase detection reaction using developer solution (36 µM 5'-bromo-4-chloro-3-indolylphosphate, 400 µM nitro blue tetrazolium, 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) at room temperature, until bands are visible.

In this way, the polyclonal antibodies according to the invention can be prepared.

Immunization protocol for polyclonal antibodies in chickens

40 µg gel-purified fusion protein are used per immunization in 0.8 ml PBS and 0.8 ml complete Freund's adjuvant and incomplete Freund's adjuvant, respectively.

day 0: 1st immunization (complete Freund's adjuvant)
day 28: 2nd immunization (incomplete Freund's adjuvant; icFA)
day 50: 3rd immunization (icFA)

Antibodies are extracted from yolk and tested in a Western blot. Polyclonal antibodies according to the invention are detected in this way.

Immunization protocol for monoclonal mouse antibodies

12 µg gel-purified fusion protein are used per immunization in 0.25 ml PBS and 0.25 ml complete Freund's adjuvant and incomplete Freund's adjuvant, respectively. The fusion protein was dissolved in 0.5 ml (without adjuvant) in the 4th immunization.

day 0: 1st immunization (complete Freund's adjuvant)
day 28: 2nd immunization (incomplete Freund's adjuvant; icFA)
day 56: 3rd immunization (icFA)
day 84: 4th immunization (PBS)
day 87: fusion

Supernatants of hybridomas are tested in a Western blot. Monoclonal antibodies according to the invention are detected in this way.

Claims

1. A protease-related protein, the protein comprising the amino acid sequence of fig. 1 or an amino acid sequence differing therefrom by one or more amino acids.

2. A DNA coding for a protein according to claim 1, wherein the DNA comprises:
   (a) the DNA of fig. 1 or a DNA differing therefrom by one or more base pairs,
   (b) a DNA hybridizing with the DNA of (a), or
   (c) a DNA related to the DNA of (a) or (b) via the degenerated genetic code.

3. An expression plasmid comprising the DNA according to claim 2.

4. A transformant containing the expression plasmid according to claim 3.

5. A process for the preparation of the protein according to claim 1, comprising the cultivation of the transformant according to claim 4 under suitable conditions.

6. Antibodies directed against the protein according to claim 1.

7. Use of the protein according to claim 1 and the DNA according to claim 2 as well as the antibody according to claim 6 for detecting the keratinization of hair.

8. Use of the protein according to claim 1 for the negative regulation of the keratinization of hair.

9. Use according to claim 8, wherein the protein is present as such or in the form of a nucleic acid expressing it.

10. Use according to claim 8 or 9, wherein substances are also used which inhibit the proteins Ha3 and/or CK15.

11. Use according to claim 10, wherein the substances are antibodies directed against Ha3 and CK15, respectively, and/or anti-sense oligonucleotides, all of which inhibit the expression of the nucleic acids encoding these proteins.

12. Use of the protein according to claim 1 for the positive regulation of the certification of hair.

13. Use according to claim 12, wherein the protein is present in the form of a substance inhibiting it.

14. Use according to claim 13, wherein the substance is an antibody according to claim 6 and/or an anti-sense oligonucleotide which inhibits the expression of the nucleic acid encoding the protein.

15. Use according to any one of claims 12 to 14, wherein the proteins Ha3 and/or CK15 are also present as such or in the form of nucleic acids expressing them.

Abstract of the Disclosure

Protease-Related Protein

The present invention relates to a protease-related protein, a DNA encoding the same and a process for the preparation thereof. Furthermore, this invention concerns the use of the DNA and the protein as well as antibodies directed against the protein and antagonistic substances.

```
5'- TAG GTG GTG TCA TTC CCC TCC AAC CTG AGT GCT GGC AGG TAC  42

                                M   P   M   K   M   L   T   M   8
ACT GCT GGC CAC CAG CAG ATG CCC ATG AAG ATG CTG ACA ATG      84

 K   M   L   A   L   C   L   V   L   A   K   S   A   W   22
AAG ATG CTG GCC CTG TGC TTG GTT CTT GCT AAA TCA GCC TGG     126

 S   E   E   Q   E   K   V   V   H   G   G   P   C   L   36
TCG GAG GAA CAG GAG AAG GTG GTT CAT GGA GGC CCG TGT TTG     168

 K   D   S   H   P   F   Q   A   A   L   Y   T   S   G   50
AAG GAC TCC CAC CCT TTC CAG GCT GCC CTC TAC ACC TCA GGT     210

 H   L   L   C   G   G   V   L   I   D   P   Q   W   V   64
CAC TTG CTG TGT GGT GGG GTC CTC ATT GAC CCA CAG TGG GTG     252

 L   T   A   A   H   C   K   K   P   N   L   Q   V   I   78
CTG ACA GCT GCC CAC TGC AAA AAA CCG AAT CTG CAG GTG ATC     294

 L   G   K   H   N   L   R   Q   T   E   T   F   Q   R   92
TTG GGG AAA CAC AAC CTA CGG CAA ACA GAG ACT TTC CAA AGG     336

 Q   I   S   V   D   R   T   I   V   H   P   R   Y   N  106
CAA ATC TCA GTG GAC AGG ACT ATT GTC CAT CCC CGC TAC AAC     378

 P   E   T   H   D   N   D   I   M   M   V   H   L   K  120
CCT GAA ACC CAC GAC AAT GAC ATC ATG ATG GTG CAT CTG AAA     420

 N   P   V   K   F   S   K   K   I   Q   P   L   P   L  134
AAT CCA GTC AAA TTC TCT AAA AAG ATC CAG CCT CTG CCC TTG     462

 K   N   D   C   S   E   E   N   P   N   C   Q   I   L  148
AAG AAT GAC TGC TCT GAG GAG AAT CCC AAC TGC CAG ATC CTG     504

 G   W   G   K   M   E   N   G   D   F   P   D   T   I  162
GGC TGG GGC AAG ATG GAA AAT GGT GAC TTC CCA< GAT ACC ATT    546

 Q   C   A   D   V   H   L   V   P   R   E   Q   C   E  176
CAG TGT GCT GAT GTC CAT CTG GTG CCC CGG GAG CAG TGT GAG     588

 R   A   Y   P   G   K   I   T   Q   S   M   V   C   A  190
CGT GCC TAC CCT GGC AAG ATC ACC CAG AGC ATG GTG TGC GCA     630

 G   D   M   K   E   G   N   D   S   C   Q   G   D   S  204
GGC GAC ATG AAA GAA GGC AAC GAT TCC TGT CAG GGT GAT TCT     672

 G   G   P   L   V   C   G   G   R   L   R   G   L   V  218
GGA GGT CCC CTA GTA TGT GGG GGT CGC CTC CGA GGG CTC GTG     714
```

Fig. 1

Fig. 1 continued

S	**W**	**G**	**D**	**M**	**P**	**C**	**G**	**S**	**K**	**E**	**K**	**P**	**G**	232
TCA	TGG	GGT	GAC	ATG	CCC	TGT	GGA	TCA	AAG	GAG	AAG	CCA	GGA	756
V	**Y**	**T**	**D**	**V**	**C**	**T**	**H**	**I**	**R**	**W**	**I**	**Q**	**N**	246
GTT	TAC	ACC	GAT	GTC	TGC	ACT	CAT	ATC	AGA	TGG	ATC	CAA	AAC	798
I	**L**	**R**	**N**	**K**	**W**	**L**								253
ATC	CTC	AGA	AAC	AAG	TGG	CTG	TGA	-3´						840

DECLARATION
AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. underneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

PROTEASE-RELATED PROTEIN

and for which a patent application:
☐ is attached hereto and includes amendment(s) filed on *(if applicable)*
☒ was filed in the United States on 18 February 2000 as Application No. *(for declaration not accompanying application)*
with amendment(s) filed on *(if applicable)*
☒ was filed as PCT international Application No. PCT/EP98/05110 on 12 August 1998 and was amended under PCT Article 19 on *(if applicable)*

I hereby state that I have reviewed and understand the contents of the above identified application, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
197 36 198.6	Germany	20 August 1997	YES ☒ NO ☐
			YES ☐ NO ☐

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS		
		PATENTED	PENDING	ABANDONED

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202
Terence N. DEAR	Thomas BOEHM
DATE 3.4.00	DATE Apr 13, 2000

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(B) STREET: Im Neuenheimer Feld 280
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(F) POSTAL CODE (ZIP): 69120

(ii) TITLE OF INVENTION: Protease-related Protein

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: PCT/EP98/05110

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: DE 197 36 198.6
(B) FILING DATE: 20-AUG-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 822 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:61..819

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:61..819

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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TAGGTGGTGT CATTCCCCTC CAACCTGAGT GCTGGCAGGT ACACTGCTGG CCACCAGCAG       60

ATG CCC ATG AAG ATG CTG ACA ATG AAG ATG CTG GCC CTG TGC TTG GTT         108
Met Pro Met Lys Met Leu Thr Met Lys Met Leu Ala Leu Cys Leu Val
  1               5                  10                  15

CTT GCT AAA TCA GCC TGG TCG GAG GAA CAG GAG AAG GTG GTT CAT GGA         156
Leu Ala Lys Ser Ala Trp Ser Glu Glu Gln Glu Lys Val Val His Gly
            20                  25                  30

GGC CCG TGT TTG AAG GAC TCC CAC CCT TTC CAG GCT GCC CTC TAC ACC         204
Gly Pro Cys Leu Lys Asp Ser His Pro Phe Gln Ala Ala Leu Tyr Thr
        35                  40                  45

TCA GGT CAC TTG CTG TGT GGT GGG GTC CTC ATT GAC CCA CAG TGG GTG         252
Ser Gly His Leu Leu Cys Gly Gly Val Leu Ile Asp Pro Gln Trp Val
    50                  55                  60

CTG ACA GCT GCC CAC TGC AAA AAA CCG AAT CTG CAG GTG ATC TTG GGG         300
Leu Thr Ala Ala His Cys Lys Lys Pro Asn Leu Gln Val Ile Leu Gly
65                  70                  75                  80

AAA CAC AAC CTA CGG CAA ACA GAG ACT TTC CAA AGG CAA ATC TCA GTG         348
Lys His Asn Leu Arg Gln Thr Glu Thr Phe Gln Arg Gln Ile Ser Val
                85                  90                  95

GAC AGG ACT ATT GTC CAT CCC CGC TAC AAC CCT GAA ACC CAC GAC AAT         396
Asp Arg Thr Ile Val His Pro Arg Tyr Asn Pro Glu Thr His Asp Asn
            100                 105                 110

GAC ATC ATG ATG GTG CAT CTG AAA AAT CCA GTC AAA TTC TCT AAA AAG         444
Asp Ile Met Met Val His Leu Lys Asn Pro Val Lys Phe Ser Lys Lys
        115                 120                 125

ATC CAG CCT CTG CCC TTG AAG AAT GAC TGC TCT GAG GAG AAT CCC AAC         492
Ile Gln Pro Leu Pro Leu Lys Asn Asp Cys Ser Glu Glu Asn Pro Asn
    130                 135                 140

TGC CAG ATC CTG GGC TGG GGC AAG ATG GAA AAT GGT GAC TTC CCA GAT         540
Cys Gln Ile Leu Gly Trp Gly Lys Met Glu Asn Gly Asp Phe Pro Asp
145                 150                 155                 160

ACC ATT CAG TGT GCT GAT GTC CAT CTG GTG CCC CGG GAG CAG TGT GAG         588
Thr Ile Gln Cys Ala Asp Val His Leu Val Pro Arg Glu Gln Cys Glu
                165                 170                 175

CGT GCC TAC CCT GGC AAG ATC ACC CAG AGC ATG GTG TGC GCA GGC GAC         636
Arg Ala Tyr Pro Gly Lys Ile Thr Gln Ser Met Val Cys Ala Gly Asp
            180                 185                 190

ATG AAA GAA GGC AAC GAT TCC TGT CAG GGT GAT TCT GGA GGT CCC CTA         684
Met Lys Glu Gly Asn Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu
        195                 200                 205

GTA TGT GGG GGT CGC CTC CGA GGG CTC GTG TCA TGG GGT GAC ATG CCC         732
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Val Cys Gly Gly Arg Leu Arg Gly Leu Val Ser Trp Gly Asp Met Pro
    210                 215                 220

TGT GGA TCA AAG GAG AAG CCA GGA GTT TAC ACC GAT GTC TGC ACT CAT      780
Cys Gly Ser Lys Glu Lys Pro Gly Val Tyr Thr Asp Val Cys Thr His
225                 230                 235                 240

ATC AGA TGG ATC CAA AAC ATC CTC AGA AAC AAG TGG CTG TGA           822
Ile Arg Trp Ile Gln Asn Ile Leu Arg Asn Lys Trp Leu
            245                 250
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 253 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Pro Met Lys Met Leu Thr Met Lys Met Leu Ala Leu Cys Leu Val
 1               5                  10                  15

Leu Ala Lys Ser Ala Trp Ser Glu Glu Gln Glu Lys Val Val His Gly
            20                  25                  30

Gly Pro Cys Leu Lys Asp Ser His Pro Phe Gln Ala Ala Leu Tyr Thr
        35                  40                  45

Ser Gly His Leu Leu Cys Gly Gly Val Leu Ile Asp Pro Gln Trp Val
    50                  55                  60

Leu Thr Ala Ala His Cys Lys Lys Pro Asn Leu Gln Val Ile Leu Gly
65                  70                  75                  80

Lys His Asn Leu Arg Gln Thr Glu Thr Phe Gln Arg Gln Ile Ser Val
                85                  90                  95

Asp Arg Thr Ile Val His Pro Arg Tyr Asn Pro Glu Thr His Asp Asn
            100                 105                 110

Asp Ile Met Met Val His Leu Lys Asn Pro Val Lys Phe Ser Lys Lys
        115                 120                 125

Ile Gln Pro Leu Pro Leu Lys Asn Asp Cys Ser Glu Glu Asn Pro Asn
    130                 135                 140

Cys Gln Ile Leu Gly Trp Gly Lys Met Glu Asn Gly Asp Phe Pro Asp
145                 150                 155                 160

Thr Ile Gln Cys Ala Asp Val His Leu Val Pro Arg Glu Gln Cys Glu
                165                 170                 175
```

```
Arg Ala Tyr Pro Gly Lys Ile Thr Gln Ser Met Val Cys Ala Gly Asp
            180                 185                 190

Met Lys Glu Gly Asn Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu
        195                 200                 205

Val Cys Gly Gly Arg Leu Arg Gly Leu Val Ser Trp Gly Asp Met Pro
    210                 215                 220

Cys Gly Ser Lys Glu Lys Pro Gly Val Tyr Thr Asp Val Cys Thr His
225                 230                 235                 240

Ile Arg Trp Ile Gln Asn Ile Leu Arg Asn Lys Trp Leu
                245                 250
```